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A Loop in Action: the Mechanism of the GDP-Mannose Mannosyl Hydrolase

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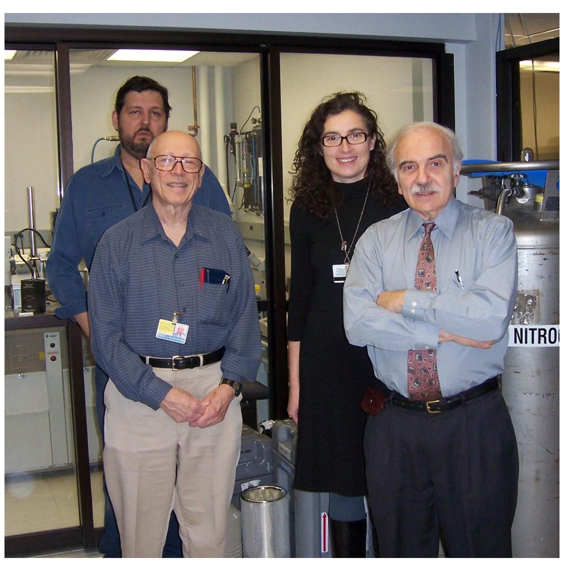
GDP-mannose hydrolase (GDPMH) catalyzes the hydrolysis with inversion of GDP-α-D-hexose to GDP and β-D-hexose by nucleophilic substitution at C1 of the hexose.

While the enzyme structure was known, little was understood about the dynamic processes that contribute to the reaction mechanism. This work identifies the motions in GDPMH involved in enzyme catalysis. We used crystal structures, and NMR studies, as well as site-directed mutagenesis and molecular modeling of the transition state to depict four stages in the catalytic cycle of this enzyme: free enzyme, substrate complex, transition state complex, and product complex.

The three structures of GDPMH – free enzyme, substrate complex, and product complex – suggest that major conformational changes take place during the hydrolysis of GDP-mannose. In the structure of the free enzyme, loop L6 spanning residues 119 to 125 is dynamically disordered (**Figure 1A**) as indicated by the lack of density in the x-ray structure and by the broadening of the imidazole ^{15}N resonance of His-124 in the HMQC spectrum (**Figure 1B**). The H124Q mutation decreases k_{cat} $10^{3.4}$-fold and largely abolishes its pH dependence. The structure of the GDPMH-Mg^{2+}-GDP-mannose complex (substrate complex), carried out using the Y103F mutant (k_{cat} two orders of magnitude lower than that of the wild type enzyme) shows that after binding Mg^{2+} and GDP-mannose, the loop becomes ordered but in an open conformation in which the catalytic base, His-124, is 12 Å away from the position required for catalysis (**Figure 1A, 2B**). This open conformation of the substrate complex appears incompatible with the catalytic requirements of the enzyme.

In the structure of the product complex, on the other hand, the loop closes onto the catalytic site, bringing His-124 into the position in which it can act as the catalytic base (closed conformation) (**Figure 1A, 2D**). The lack of the tyrosine-OH group could prevent the bound substrate from triggering the conformational change to that observed in the product complex. However, there is no direct involvement of the tyrosine-OH in stabilizing the closed conformation. A better explanation is that the partial positive charge developing on C1 of the mannose during hydrolysis causes loop L6 to close. A closer look at the structure of the product complex supports this hypothesis, since the product structure contains a cationic Tris molecule bound in the catalytic site in the position occupied by the substrate mannose in the substrate complex. The hydroxyl groups of the Tris molecule make hydrogen bonds with side chains of the same residues that bind the mannose in the substrate complex. Hence, it appears that closing of the negatively charged catalytic loop L6 (which contains Asp-121, Glu-122, and Asp-125) is driven by the positive charge that is developing on C1 of the mannose. In the Y103F mutant, the loop L6 does not close because the hydroxyl of Tyr-103, which makes a hydrogen bond to the leaving oxygen atom of the β-phosphate, is probably required for the charge separation that takes place in the partially dissociative mechanism.



Authors (from left) Mario A. Bianchet, Albert S. Mildvan, Sandra B. Gabelli, and L. Mario Amzel

The group's research was showcased on the cover of the October 2, 2006 edition of Biochemistry.

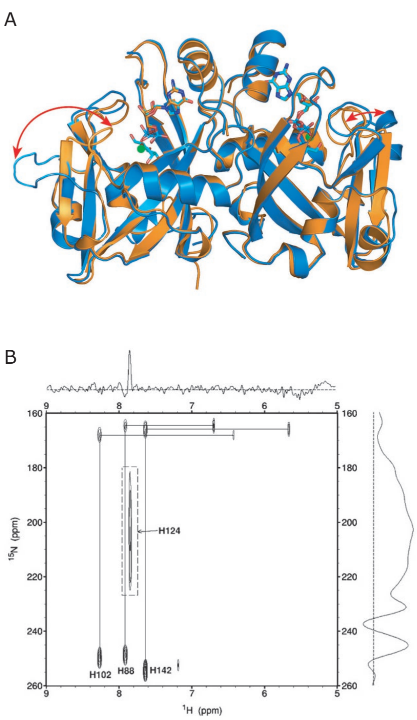


***Figure 1**. Dynamics in the mechanism of GDPMH. (A) Structural Superposition of the crystal structures of the closed product complex of GDPMH (orange), and the open substrate complex of the Y103F mutant enzyme (blue). The catalytic metals are shown as green spheres. The structures show the amplitude of motion of the catalytic loop L6 between the closed (active) and open state (inactive). (B) 2D ^{1}H-^{15}N HMQC spectrum of the imidazole region of free, wild type GDPMH at 30°C and pH 8.3. Note the broadening of the imidazole ^{15}N resonance of His-124.*

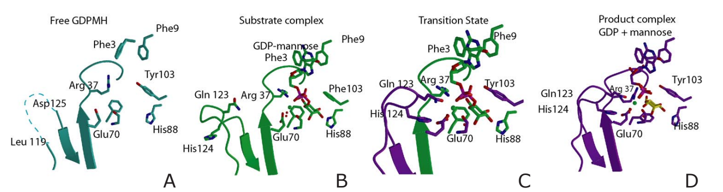


***Figure 2**. Snapshots of the catalytic cycle of GDPMH. (A) Crystallographic structure of the free enzyme. In this structure, loop L6 (dotted line) is disordered at the beginning of the cycle (pdb id 2GT2). (B) X-ray structure of the substrate complex. In this structure, although loop L6 becomes ordered, it remains in an "open" conformation (PDB id 1GT4). (C) Model of the GDPMH-transition state complex. This model was obtained by combining the coordinates of the enzyme in the product structure with the position of the GDP-mannose in the substrate structure (PDB id 2GT4) distorting the mannose to a half chair and elongating the mannosyl C1-GDP bond. In this model, loop L6 is in the "closed" conformation, bringing residues Gln123 and His 124 into their active positions. This conformational change is probably triggered by the positive charge developing on C1 of the mannose in the partially dissociative transition state acting on the negative charges in loop L6 (Asp-121, Glu-122, and Asp-125). (D) Product complex. This structure corresponds to the stage in the catalytic cycle before the release of products (PDB id 1RYA).*